2.2. Background Detection

We assume that the total fluorescent emitted from a target location consists of two components: fluorescent signal derived from fluor-tagged mRNA specific to the target, and background fluorescent due to non-specific binding to the glass surface, or the target itself. We choose to use the peripheral area of the target under consideration to estimate the fluorescent background at the target site if no signal is presented. In Figure 2(a), a region surrounding the target is selected. If the size of the inner region is less than 40 pixels, then it is enlarged to 40 pixels to guarantee a sufficient number of pixels. A histogram is then constructed, shown in Figure 2(b), where the mode represents where the fluorescent background is. We choose the mode of the histogram instead of the mean because of the perturbation of the right side of the histogram by the signal. It is important to note that the left side of the histogram reflects the normal distribution nature of the fluorescent background with minimum perturbation from the target. To utilize these characteristics of the fluorescent background, an estimation procedure is summarized as follows,

(i) Average across each row or column to produce two projections as shown in Figure 2(a). Find the minimum value, v, near the border of the target (or at 4 corners). We limit to the border region because the center of the target may actually be darker than the background.

(ii) Take v as the initial estimate of the mean, μ_b, of the background. Take the gray-level, g, above 1 percentile within the target region (the left side of the histogram without including the center of the target) to be the range spanned by $3\sigma_b$ to the right side of the histogram, or $\sigma_b = g/3$.

(iii) Re-estimate μ_b and σ_b from within the initial range $(\mu_b - 3\sigma_b, \mu_b + 3\sigma_b) = (v - g, v + g)$. The mean μ_b is further refined within the range $(\mu_b - \sigma_b, \mu_b + \sigma_b)$, and it is then taken as the estimate of the local background.

The procedure is designed to avoid several problems: a possibly darker center hole of the target, the heavier tailor of the histogram to the right side, and the possibility of two peaks in the background mode estimation. It is not necessary to know where the target is within each target region in order to extract the statistics of the background. Instead, these statistics provide essential information for target detection in following stages.

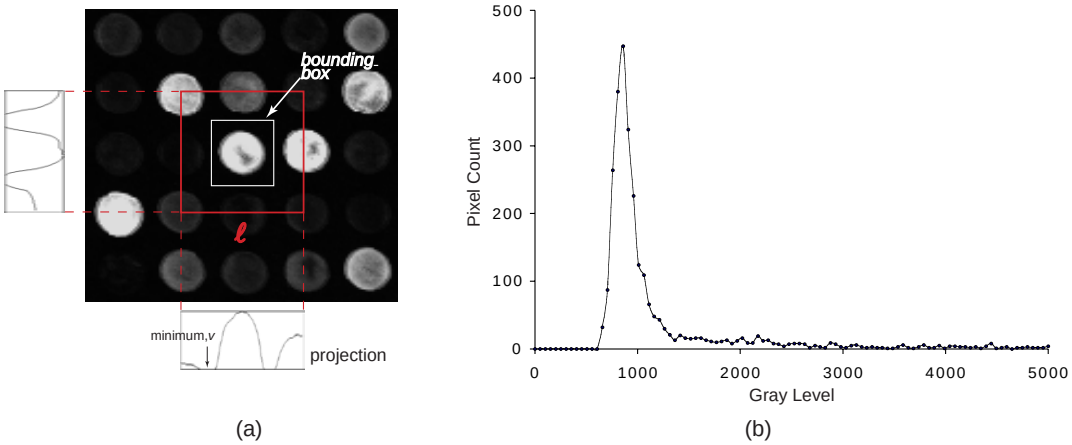

Figure 2. Background extraction.